

CLINICAL-ALIMENTARY TRACT

A Randomized, Double-Blind, Placebo-Controlled Trial of Fluticasone Propionate for Pediatric Eosinophilic Esophagitis

MICHAEL R. KONIKOFF,* RICHARD J. NOEL,[†] CARINE BLANCHARD,[§] CASSIE KIRBY,[§] SEAN C. JAMESON,[§] BRIDGET K. BUCKMEIER,[§] RACHEL AKERS,^{||} MITCHELL B. COHEN,* MARGARET H. COLLINS,[¶] AMAL H. ASSA'AD,[§] SEEMA S. ACEVES,[#] PHILIP E. PUTNAM,* and MARC E. ROTHENBERG[§]

*Division of Pediatric Gastroenterology, Hepatology and Nutrition, [§]Division of Allergy and Immunology, ^{||}Center for Epidemiology and Biostatistics, and [¶]Division of Pathology and Laboratory Medicine, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, Ohio; [†]Division of Pediatric Gastroenterology and Nutrition, Department of Pediatrics, Children's Hospital of Wisconsin, Medical College of Wisconsin, Milwaukee, Wisconsin; and [#]Division of Allergy and Immunology, Department of Pediatrics, Children's Hospital of San Diego, University of California San Diego, La Jolla, California

See editorial on page 1629; CME Quiz on page 1625.

Background & Aims: Eosinophilic esophagitis is an increasingly recognized disorder with distinctive endoscopic, histologic, and allergic features. Although several therapies are advocated, no placebo-controlled trials have been conducted. We aimed to determine the efficacy of swallowed fluticasone propionate (FP) in the treatment of eosinophilic esophagitis. **Methods:** We conducted a randomized, double-blind, placebo-controlled trial of swallowed FP in pediatric patients with active eosinophilic esophagitis. Thirty-six patients were randomly assigned to receive either 880 μ g of FP (21 patients) or placebo (15 patients) divided twice daily for 3 months. The primary end point was histologic remission, defined by a peak eosinophil count of ≤ 1 eosinophil in all 400 $\times$ fields in both the proximal and distal esophagus. **Results:** Fifty percent of FP-treated patients achieved histologic remission compared with 9% of patients receiving placebo ($P = .047$). FP decreased esophageal eosinophil levels, with a more pronounced effect in nonallergic individuals (65.9 ± 25.3 vs 1.4 ± 1.1 eosinophils/high-power field in the proximal esophagus [$P = .03$] and 84.6 ± 19.7 vs 19.6 ± 12.9 eosinophils/high-power field in the distal esophagus [$P = .04$]). Resolution of vomiting occurred more frequently with FP than placebo (67% vs 27%; $P = .04$). FP-induced resolution of mucosal eosinophilia was associated with resolution of endoscopic findings, epithelial hyperplasia, younger age ($P = .0003$), shorter height ($P = .002$), and lighter weight ($P = .02$). Effective treatment with FP decreased the number of CD8⁺ T lymphocytes and mast cells in both the proximal and distal esophagus ($P < .05$). **Conclusions:** Swallowed FP is effective in inducing histologic remission in eosinophilic esophagitis, with a more pronounced effect in nonallergic and younger individuals, especially in the proximal esophagus.

Eosinophilic esophagitis (EE) is an inflammatory disorder characterized by the accumulation of eosinophils in the esophageal epithelium and a range of clinical symptoms including abdominal pain, vomiting, dysphagia, food impaction, and

food refusal.^{1,2} Although the clinical presentation of patients with EE can mimic gastroesophageal reflux disease, patients with EE typically have normal findings on esophageal pH probe studies and do not respond to standard therapies for gastroesophageal reflux disease.³⁻⁵ Patients with EE often display characteristic endoscopic findings including furrowing, rings, or exudates in both the proximal and distal esophagus.⁶⁻⁸ Distinctive histopathologic features are also found in EE, including the presence of large numbers of eosinophils (typically ≥ 24 /high-power field [HPF]) in esophageal squamous epithelium, epithelial hyperplasia (as assessed histologically and by staining for the Ki-67 antigen), layering of eosinophils on the surface of the mucosa, and, in some cases, superficial eosinophilic microabscesses.^{2,9,10} Several lines of evidence support an allergic cause, including the high incidence of atopy in patients with EE,¹¹ the improvement in symptoms and histology with dietary antigen restriction,^{12,13} the characterization of a T_H2-type inflammatory response in esophageal biopsy specimens,¹⁴ and the induction of experimental EE by allergen challenge in animal models.^{15,16}

Previous case series have documented effective therapies for EE, including elimination and elemental diets as well as topical and systemic corticosteroids, but controlled clinical trials have not yet been conducted. Directed elimination diets based on the results of skin prick and/or skin patch testing have variable efficacy, perhaps because current strategies may not be optimal for identifying both immunoglobulin E- and cell-mediated phenomena.^{11,12,17} Based on clinical experience, elemental diets consisting of exclusive intake of an amino acid-based formula appear to be effective in inducing remission in EE but are unpalatable and costly and often require nasogastric or gastrostomy tube administration, which limits their widespread use.^{13,18,19} Systemic corticosteroids appear to be effective at inducing remission in EE, but their long-term use is limited by side effects including growth impairment, adrenal suppression, reduced bone density, and cataracts.²⁰ In 1998, Faubion et al

Abbreviations used in this paper: EE, eosinophilic esophagitis; EGD, esophagogastroduodenoscopy; FP, fluticasone propionate; HPF, high-power field.

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reported the successful use of a topical glucocorticoid, fluticasone propionate (FP), puffed from a metered-dose inhaler and swallowed for the treatment of EE.²¹ Since then, retrospective or uncontrolled studies have shown that swallowed FP therapy is associated with disease remission.^{8,22,23} A recent study of 20 patients found that allergic individuals were relatively refractory to FP therapy compared with nonallergic patients with EE,²³ but this and other studies are limited by their retrospective nature and the lack of a placebo group. Because of its low oral bioavailability and rapid first-pass metabolism in the liver, FP has a favorable side effect profile relative to systemic glucocorticoids.²⁴ Based on the potential value of FP in the treatment of EE, we conducted a double-blind placebo-controlled clinical trial to evaluate the efficacy of FP compared with placebo in patients with EE. Additionally, we aimed to determine the clinical and pathologic features associated with disease remission.

Materials and Methods

Selection of Patients

The study was performed between January 10, 2003, and August 16, 2005, at Cincinnati Children's Hospital Medical Center. Two patients were also enrolled at Children's Hospital, San Diego. Eligible patients were between the ages of 3 and 30 years with a diagnosis of EE confirmed histologically. Specifically, EE was defined as the presence of ≥ 24 eosinophils in any 400 $\times$ HPF in at least one biopsy specimen from either the proximal or distal esophagus and the presence of epithelial hyperplasia after careful examination of all microscopic fields. Prior acid suppression therapy was not necessary for the diagnosis of EE. Patients were excluded from the study if they had a history of poor tolerance to FP, were unable to cooperate with the use of a metered-dose inhaler, were pregnant, or had taken any corticosteroid (including inhaled, nasal, or systemic) within 3 months. The institutional review board at each center approved the study, and all participants (or their parents or guardian) gave written informed assent/consent.

Study Design

This 3-month study was a randomized, double-blind, placebo-controlled trial designed to determine the efficacy and safety of swallowed FP in the treatment of patients with EE. Eligible patients underwent an allergy evaluation that included skin prick testing to both food and aeroallergens. If food allergens were identified, the patient was offered a 3-month elimination diet involving restriction of these foods. If the elimination diet was successful as defined by histologic remission on repeat endoscopy performed at the end of the elimination diet, patients were not enrolled in the trial. After refusal or failure of the 3-month elimination diet (as defined by persistent EE on repeat endoscopy), or if no food allergies were identified, patients were randomly assigned to receive either swallowed FP (880 μ g/day) or placebo from a metered-dose inhaler divided twice daily for approximately 3 months. Two thirds of the patients were assigned to FP, and one third were assigned to placebo. After completing the prescribed therapy, patients underwent an esophagogastroduodenoscopy (EGD) with biopsies of the proximal and distal esophagus. Each patient's diet, as well as all other medications including acid suppression therapy, was continued unchanged for the duration of the study.

A clinical research coordinator dispensed metered-dose inhalers containing either active drug or placebo to each patient according to a computer-generated randomization list. No blocking or stratification schemes were used. All participants and study personnel (with the exception of the above clinical research coordinator) were blinded to treatment assignment for the duration of the study. Only the study statisticians had access to the unblinded data, but they did not have contact with study participants.

Clinical symptom information was collected at study enrollment and at each patient's follow-up EGD for the final 10 patients enrolled in the study. Recorded symptoms included nausea, vomiting, abdominal pain, chest pain, heartburn, regurgitation, dysphagia, food impaction, poor appetite, early satiety, fussiness (for nonverbal patients), and poor weight gain. Retrospective clinical symptom information was obtained by chart review when available for the remainder of the patients in the study. A detailed endoscopic assessment of each patient's esophagus at prestudy and follow-up EGD was obtained from the hospital EGD operative report generated by each patient's treating gastroenterologist. Verbal assurance of compliance with the treatment medication was obtained from each patient or his or her parent or guardian. In addition, the final 10 patients enrolled in the study were given a calendar at entry and instructed to record the time of each medication dose. Examination of calendars collected at follow-up EGD showed complete compliance.

Skin Prick Testing

Skin prick tests were performed with commercially available extracts to a variety of food antigens and common aeroallergens (Greer Laboratories, Lenoir, NC; Hollister-Stier, Spokane, WA; and ALK-Abelló, Hørsholm, Denmark), and those patients with any positive skin prick test results to food and/or environmental allergens were considered allergic. The allergen extracts were applied intracutaneously with a GreerPick device (Greer Laboratories). Reactivity was determined after 15 minutes by measuring the wheal-and-flare response compared with a positive histamine control and a negative control saline. Positive reactivity was defined as reactions unequivocally greater than the saline control. Aeroallergen extracts included cat, cockroach, dog, grass, mite mix, mold mix, ragweed, tree mix, and weed mix. Food extracts included almond, apple, apricot, avocado, banana, barley, bean (string), beef, blueberry, Brazil nut, broccoli, cantaloupe, carrot, casein, cashew, celery, cherry, chicken, cinnamon, coconut, codfish, corn, egg white, egg yolk, flounder, grape (white), halibut, hazelnut, lamb, lemon, lettuce, lobster, milk (cow's), mustard, oat, orange, pea, peach, peanut, pear, pecan, pistachio, pork, potato (sweet), potato (white), raspberry, rice, rye, scallops, sesame, shrimp, soy, spinach, squash, strawberry, sunflower, tomato, tuna, turkey, walnut (English), watermelon, and wheat.

Medication

Patients were treated with swallowed FP or placebo (kindly provided by GlaxoSmithKline, Research Triangle Park, NC) between 2 endoscopic assessments. Patients were given identical metered-dose inhalers of FP or placebo. They were instructed not to use a spacer. Additionally, they were instructed to spray the medication into the pharynx and not to rinse, eat, or drink for 30 minutes after administration. All

patients and/or guardians expressed understanding of instructions and assured compliance.

Analysis of Histologic Specimens

Multiple grasp biopsy specimens were obtained at the discretion of the endoscopist from the proximal and distal esophagus as well as the stomach and duodenum. Five-micrometer sections were cut from routinely processed, formalin-fixed, paraffin-embedded tissue blocks and were stained with H&E. Slides were examined in random order by a blinded investigator. Intraepithelial eosinophils in all 400× HPFs were counted in one histologic section of each biopsy specimen, and the peak eosinophil count for each biopsy was defined as the maximum number of eosinophils in any single HPF. An HPF was counted only if at least half of the field was occupied by tissue. An average of 16.3 ± 6.9 (mean $\pm$ SD) HPFs were assessed per specimen. All slides were also assessed for layering of eosinophils on the mucosal surface and the presence of epithelial hyperplasia. The thickness of the basal layer in relation to the entire epithelium was estimated in well-oriented sections, and epithelial hyperplasia was graded on a 4-point scale: grade 0, no hyperplasia; grade 1, basal layer occupying less than one third of the total epithelial thickness; grade 2, basal layer occupying one third to two thirds of the total epithelial thickness; and grade 3, basal layer occupying more than two thirds of the total epithelial thickness. Biopsy specimens from the stomach and duodenum were assessed to ensure they were free of significant disease. Esophageal biopsy specimens with adequate tissue were stained with MIB-1 (Ki-67) antibody (predilute, Dako, Carpinteria, CA) to detect proliferating cells, CD8 antibody (predilute, Cell Marque, Hot Springs, AK) to detect cytotoxic T lymphocytes, and tryptase antibody (predilute, Cell Marque) to detect mast cells. All antibodies were used according to the manufacturer's instructions. Nuclei staining with MIB-1 antibody in 300 contiguous nuclei in well-oriented areas of each specimen were counted, and the MIB-1 index was calculated as the number of stained nuclei per 100 nuclei. CD8⁺ T lymphocytes and mast cells in up to ten 400× HPFs in well-oriented areas of each specimen were counted.

Definition of Disease Remission

Patients were considered to have achieved remission if they had complete histologic response to treatment as defined by a peak eosinophil count of ≤ 1 eosinophil in all 400× HPFs in both the proximal and distal esophagus, as reported previously by our group.²³ Partial response was defined as a peak eosinophil count of >1 and <24 eosinophils per 400× HPF in both the proximal and distal esophagus. This strict definition of disease remission was used to accurately phenotype patients in this study. However, we also examined response to treatment using other definitions of disease remission to determine if redefining remission using other criteria would influence the results of the study. These redefinitions included using a higher peak eosinophil count (6 eosinophils/HPF), mean eosinophil count (defined as the mean of the eosinophil counts for all HPFs in either the proximal or distal esophagus) of ≤ 1 or ≤ 2 eosinophils/HPF, and percent reduction in peak eosinophil count from pretreatment to posttreatment (90% or 95% reduction).

Sample Size

The decision to enroll 20 patients in the FP group and 10 patients in the placebo group was based on the assumption that 10% of the group receiving placebo would respond to treatment (primary outcome measure). Sample size was based on detection of at least a 60% difference in the overall response rates between the placebo and FP treatment groups with 80% power at the .05 level of significance. An interim analysis was performed after 15 patients had been enrolled in the study.

Outcomes and Statistical Analysis

The primary outcome measure, as specified before the study was initiated, was complete histologic response to treatment as defined by a peak eosinophil count of ≤ 1 eosinophil in all 400× HPFs in both the proximal and distal esophagus. Secondary outcome measures included presence of endoscopic furrowing, presence of epithelial hyperplasia, and presence of clinical symptoms. Response to treatment was compared in the FP and placebo groups using Fisher exact test. Pretreatment and posttreatment groups were compared with respect to cell counts (paired Student *t* test), epithelial hyperplasia (Wilcoxon matched pairs test), layering of eosinophils at the mucosal surface, and endoscopic furrowing (exact McNemar's test). Baseline characteristics of the FP and placebo groups were compared, including age, sex, height, weight, body mass index, allergic status, duration of symptoms, duration of disease, previous diagnosis of asthma, and therapy (proton pump inhibitors, other acid suppression agents, and montelukast). Subgroup analysis of the preceding variables was also performed on FP responders versus nonresponders. Fisher exact tests were used to compare the distributions of dichotomous variables. Two sample Student *t* tests and Pearson correlations were used for continuous variables. The data are expressed as mean $\pm$ SE. All tests were 2 sided, and *P* values $<.05$ were considered statistically significant. Statistical tests were performed using SAS version 8.1 (SAS Institute, Inc, Cary, NC) and GraphPad Prism version 4.01 (GraphPad Software, Inc, San Diego, CA).

Results

Patient Characteristics

Ninety-two patients were screened for the study; 56 were excluded, and 36 were randomized (Figure 1). Of the 56 excluded patients, 21 did not meet entry criteria and 35 refused to participate in the placebo-controlled study. Of the 36 randomized patients, 21 received FP and 15 received placebo. Thirty-one patients completed the study. One patient was excluded from the FP group after randomization, because this patient did not meet the inclusion criteria for the diagnosis of EE. Four patients withdrew from the placebo group: 3 for increased symptoms and one for noncompliance. These 5 patients were excluded from the analysis, because they did not have follow-up esophageal biopsy specimens on which to base their response to treatment. Of the 36 randomized patients, 22 had no food allergies identified on skin prick testing and were directly enrolled in the study. Thirteen patients had food allergies identified; 4 failed and 8 refused an elimination diet, while one patient with a positive skin test result only to peanuts was directly enrolled in the trial. One additional patient who withdrew from the study after randomization did not have results of allergy testing available. Two patients had resolution of EE with

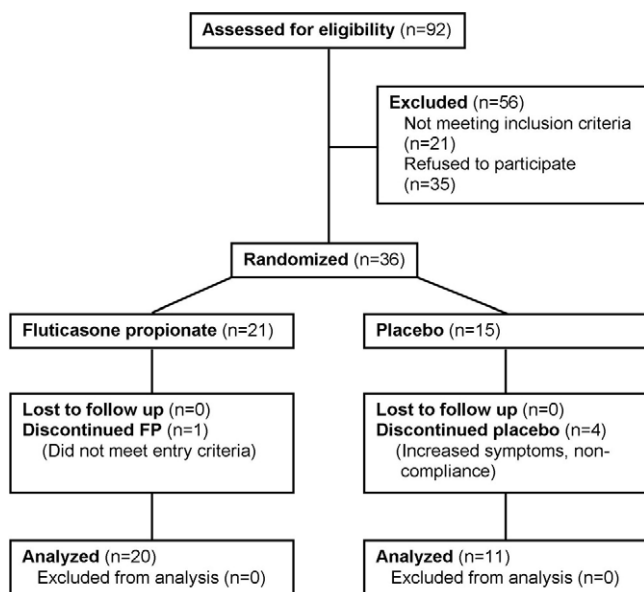


Figure 1. Flow diagram of study participants.

elimination diets before randomization. There were no significant differences between the FP and placebo groups in any of the baseline demographic or disease characteristics (Table 1). Most patients were newly diagnosed with EE in the 6 months before study enrollment (mean time from initial EE diagnosis to study enrollment was 4.5 ± 1.3 months).

Clinical Efficacy

FP improves the primary outcome, disease remission, as defined by esophageal eosinophil levels. The response rate (with remission defined as a peak eosinophil count of ≤ 1 eosinophil/HPF) was significantly greater in patients treated with FP (10/20 [50%]) compared with placebo (1/11 [9%]) ($P = .047$) (Figure 2). We aimed to determine if redefining responsiveness with different histologic criteria, including a higher peak eosinophil count, mean eosinophil count, and percent reduction in eosinophils, would also uncover a positive effect of FP compared with placebo (Figure 2 and Table 2). Although there was a trend toward improved response to FP with all redefinitions, statistical significance was achieved only with remission redefined as a mean eosinophil count of ≤ 1 eosinophil/HPF.

In the FP group as a whole, there was a trend toward reduced esophageal eosinophil levels in both the proximal and distal esophagus after treatment with FP (69.4 ± 14.5 pretreatment vs 35.2 ± 12.6 posttreatment eosinophils/HPF in the proximal esophagus [$P = .05$] and 82.2 ± 11.7 pretreatment vs 45.7 ± 13.6 posttreatment eosinophils/HPF in the distal esophagus [$P = .07$]). Patients generally had either complete histologic resolution of esophageal eosinophilia or no reduction; there were only 3 patients in the FP group who had posttreatment eosinophil counts between 1 and 24 eosinophils/HPF in either the proximal or distal esophagus (Figure 3 and Table 2).

There were no statistical differences between FP responders and nonresponders in mean pretreatment eosinophil counts in either the proximal ($P = .76$) or distal esophagus ($P = .43$). Treatment with FP trended toward greater efficacy in the prox-

imal esophagus, because 14 of 20 patients (70%) had a complete ($n = 12$) or partial ($n = 2$) response compared with 10 of 19 patients (53%) with a complete or partial response in the distal esophagus. Of note, 5 of 11 patients (45%) in the placebo group had either a complete or partial response in the proximal esophagus compared with 2 of 11 patients (18%) with a complete or partial response in the distal esophagus.

Secondary Outcomes

FP improves endoscopic and histologic parameters of EE. After treatment, significantly fewer individuals in the FP group had endoscopic distal esophageal furrowing compared with the placebo group (50% vs 91%; $P = .047$). Endoscopic distal esophageal furrowing was not present in any FP responders (0/10) after treatment, while all FP nonresponders (10/10) had persistent furrowing in the distal esophagus ($P < .0001$).

In the FP group as a whole, treatment with FP significantly reduced the layering of eosinophils on the mucosal surface in both the proximal and distal esophagus (70% pretreatment vs 30% posttreatment in the proximal esophagus [$P = .046$] and 80% pretreatment vs 40% posttreatment in the distal esophagus [$P = .02$]). These effects were more pronounced in those who responded to FP. In the distal esophagus, FP responders had significantly less surface layering of eosinophils posttreatment than FP nonresponders (0% vs 80%; $P = .0007$) or patients who received placebo (0% vs 55%; $P = .01$).

FP reduces epithelial hyperplasia. Treatment with FP significantly reduced epithelial hyperplasia in both the proximal and distal esophagus, as assessed by histologic examination of H&E-stained sections (proximal esophagus, $P = .002$; distal esophagus, $P = .01$) (Figure 4). Treatment with placebo had no effect. The reduction in epithelial hyperplasia was limited to FP responders, because FP nonresponders had no change in epithelial hyperplasia. Immunohistochemical analysis with MIB-1 (monoclonal anti-Ki-67)²⁵ (Figure 4) showed a reduction in epithelial hyperplasia in FP responders, as evi-

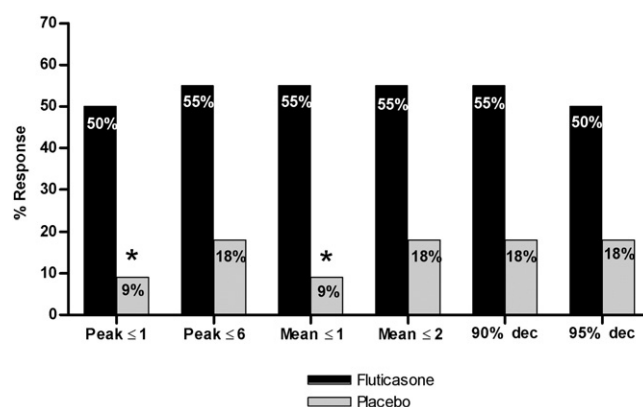


Figure 2. Percent of patients achieving response. Response (pre-specified during study design) was defined as a peak eosinophil count of ≤ 1 eosinophil/HPF in both the proximal and distal esophagus. Response rates using different definitions of remissions were also calculated to determine if these influenced response rates. These included a higher peak eosinophil count (6 eosinophils/HPF), mean eosinophil count of ≤ 1 or ≤ 2 , and percent reduction in peak eosinophil count from pretreatment to posttreatment (90% or 95% decrease). * $P < .05$.

Table 1. Baseline Characteristics of the Patients

Variable	Fluticasone	Placebo	P
Gender, male/female (% male)	17/4 (81%)	9/6 (60%)	.26
Caucasian	21 (100%)	15 (100%)	1
Mean age (SE, range) at entry (y)	8.5 (0.8, 3–16)	11.2 (1.3, 3–18)	.07
Height (cm)	131.1 ± 4.9	144.9 ± 7.9	.13
Weight (kg)	33.4 ± 4.4	44.6 ± 5.8	.13
Allergic (SPT +)	11 (52%)	7 (50%) ^a	1
Food allergic (Food SPT +)	8 (38%)	6 (43%) ^a	1
Aeroallergen allergic (Aeroallergen SPT +)	7 (33%)	5 (36%) ^a	1
History of asthma	9 (43%)	5 (33%)	.73
PPI use during study	8 (38%)	5 (33%)	1
Acid suppression (PPI or H2-RA)	10 (48%)	7 (47%)	1
Montelukast use	4 (19%)	0 (0%)	.12
Time from onset of symptoms (y)	2.8 ± 0.5	1.8 ± 0.3	.09
Time from EE diagnosis (y)	0.4 ± 0.1	0.3 ± 0.2	.75
Time to repeat endoscopy (mo)	3.4 ± 0.2	3.2 ± 0.1	.35
Peak Eos/HPF proximal esophagus	69.4 ± 14.5 ^a	72.8 ± 16.2 ^b	.88
Peak Eos/HPF distal esophagus	78.3 ± 11.7 ^b	73.7 ± 10.6	.78

SPT, skin prick test; PPI, proton pump inhibitor; H2-RA, histamine-2 receptor antagonist; HPF, high power field.

^aData missing for one patient.

^bData missing for two patients.

Table 2. Individual Patient Data and Eosinophil Counts

Pt	Age ^a	Sex	SPT	Rx ^b	Pre-Treatment								Post-Treatment							
					Proximal Esophagus				Distal Esophagus				Proximal Esophagus				Distal Esophagus			
					Peak ^c	Mean ^d	Median ^e	HPF ^f	Peak	Mean	Median	HPF	Peak	Mean	Median	HPF	Peak	Mean	Median	HPF
1	3.8	F	Neg	FP	264	103	90	17	174	87	65	13	0	0	0	21	0	0	0	19
2	4.2	M	Neg	FP	41	20	19	19	25	8	7	19	0	0	0	6	0	0	0	28
3	9.3	M	F,A	FP	28	6	1	17	51	30	25	14	54	15	6	25	54	38	39	12
4	15.7	F	Neg	P	134	16	1	12	70	36	39	20	4	1	0	21	105	34	33	17
5	3.5	M	Neg	FP	4	0	0	25	56	22	23	19	0	0	0	9	0	0	0	21
6	16.2	M	A	FP	136	30	15	18	175	86	79	14	115	14	0	19	128	78	78	13
7	15.4	M	A	FP	45	18	14	19	47	22	23	25	129	69	70	17	156	71	65	31
8	7.8	M	F	FP	106	31	4	7	135	65	58	21	0	0	0	27	0	0	0	14
9	6.0	F	A	P	65	35	37	17	94	40	31	20	ND	ND	ND	ND	ND	ND	ND	ND
10	6.6	F	F	FP	91	55	48	5	105	37	35	19	0	0	0	20	0	0	0	8
11	3.9	M	Neg	FP	90	35	34	16	72	29	23	24	0	0	0	15	0	0	0	20
12	14.2	M	F	FP	15	2	1	25	68	24	15	31	0	0	0	20	4	1	1	18
13	6.8	M	A	FP	1	0	0	18	31	11	9	15	0	0	0	15	0	0	0	26
14	14.5	F	ND	P	8	1	1	31	47	15	14	11	ND	ND	ND	ND	ND	ND	ND	ND
15	5.8	M	F,A	FP	ND	ND	ND	ND	8	1	ND	14	ND	ND	ND	ND	ND	ND	ND	ND
16	13.1	M	Neg	FP	48	15	10	19	57	31	30	26	3	1	0	18	67	23	19	22
17	12.7	M	F,A	P	62	26	22	15	23	7	5	8	275	185	153	3	107	63	79	8
18	7.1	F	Neg	P	30	4	2	18	81	26	22	16	34	15	16	5	31	11	7	8
19	11.0	M	F,A	FP	117	41	24	11	100	41	40	15	152	77	66	11	163	77	53	8
20	5.0	M	F,A	P	56	38	38	4	105	56	61	13	11	5	5	10	38	13	14	14
21	3.3	M	F	P	41	24	19	7	145	63	52	16	89	42	36	12	73	48	46	6
22	10.9	F	F	FP	128	58	54	30	48	14	1	23	141	65	60	15	130	62	54	18
23	8.4	M	Neg	FP	31	7	5	17	ND	ND	ND	ND	0	0	0	14	53	24	20	17
24	17.2	M	Neg	P	ND	ND	ND	ND	44	8	1	29	ND	ND	ND	ND	ND	ND	ND	ND
25	8.8	M	Neg	FP	130	107	ND	3	65	20	ND	11	11	7	ND	5	89	41	ND	5
26	6.1	M	F,A	FP	61	20	10	12	43	18	9	12	98	58	57	11	24	15	18	4
27	8.0	M	Neg	FP	32	14	14	12	ND	ND	ND	ND	0	0	0	20	ND	ND	ND	ND
28	16.9	M	F	P	54	19	17	21	22	9	9	11	62	22	17	15	29	3	1	24
29	9.3	F	Neg	FP	0	0	0	12	170	80	84	17	0	0	0	22	0	0	0	27
30	12.4	M	F,A	P	54	27	27	13	63	28	21	16	167	46	38	22	87	25	14	19
31	3.8	F	Neg	P	ND	ND	ND	ND	74	38	45	7	6	2	1	7	2	0	0	9
32	18.6	F	Neg	P	75	25	ND	10	102	58	ND	8	0	0	0	7	0	0	0	11
33	10.0	M	Neg	P	96	37	27	21	25	11	8	16	ND	ND	ND	ND	ND	ND	ND	ND
34	5.3	M	Neg	FP	19	4	2	22	58	22	21	14	0	0	0	22	1	0	0	24
35	11.9	M	F,A	P	237	133	122	18	155	74	76	19	64	27	24	23	85	55	55	13
36	13.6	M	Neg	P	34	16	13	11	55	20	19	32	2	0	0	26	51	19	18	30

ND, not determined; SPT, skin prick tests; FP, fluticasone propionate; P, placebo; HPF, high power field; F, positive skin prick tests to foods; A, positive skin prick tests to aeroallergens; Neg, all skin prick tests negative.

^aAge in years.

^bTreatment group, either FP or placebo.

^cPeak eosinophil count in all HPFs examined.

^dMean of the eosinophil counts for all HPFs examined in each specimen.

^eMedian of the eosinophil counts for all HPFs examined in each specimen.

^fNumber of HPFs examined in each biopsy specimen.

denced by a decreased MIB-1 index after treatment (15.2 ± 3.0 pretreatment vs 6.8 ± 1.4 posttreatment immunopositive nuclei/100 nuclei in the proximal esophagus [$P = .03$] and 13.6 ± 3.4 pretreatment vs 6.1 ± 1.8 posttreatment immunopositive nuclei/100 nuclei in the distal esophagus [$P = .005$]). There was no difference in the MIB-1 index before and after treatment in the FP group as a whole or in FP nonresponders.

FP improves vomiting. The most common clinical symptoms at the start of the study were abdominal pain (reported in 16/28 patients for whom symptom information was available [57%]), vomiting (15/28 [54%]), and dysphagia (13/29 [45%]). Only vomiting improved significantly with treatment with FP (67% pretreatment vs 27% posttreatment; $P = .04$). All patients who responded histologically had a concurrent resolution of their vomiting (6/6), while vomiting in FP nonresponders did not resolve (0/4).

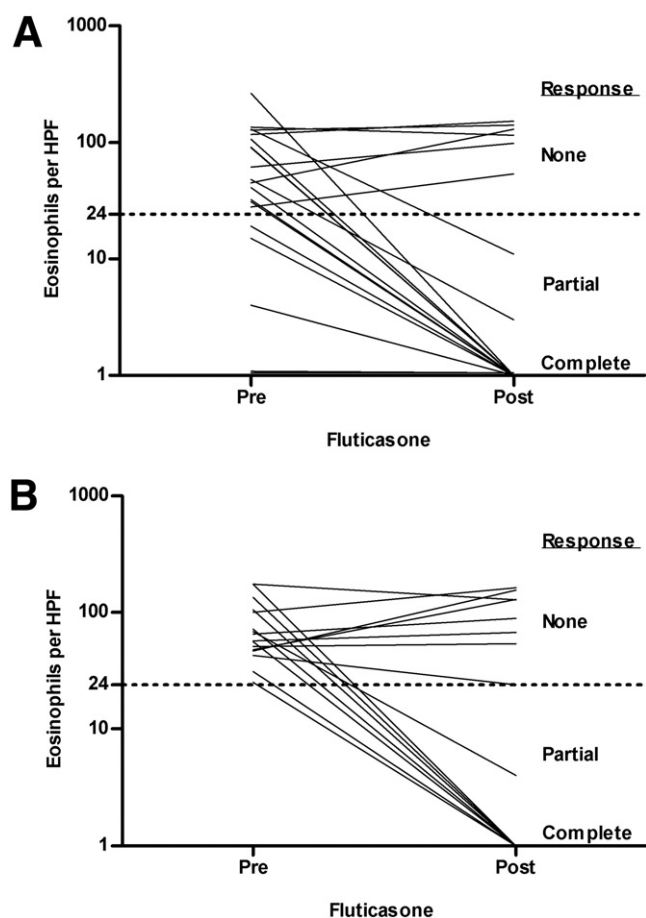


Figure 3. Effect of FP therapy on esophageal eosinophil levels in the (A) proximal and (B) distal esophagus. Response to FP was nearly complete or absent; only 3 patients had intermediate eosinophil levels (between 1 and 24 eosinophils/HPF) after treatment with FP.

Modifying Factors

FP responsiveness is higher in nonallergic individuals. With FP treatment, nonallergic patients had a significant reduction in esophageal eosinophilia in both the proximal and distal esophagus (65.9 ± 25.3 pretreatment vs 1.4 ± 1.1 posttreatment eosinophils/HPF in the proximal esophagus [$P = .03$] and 84.6 ± 19.7 pretreatment vs 19.6 ± 12.9 posttreatment eosinophils/HPF in the distal esophagus [$P = .04$]) and had significantly lower posttreatment esophageal eosinophil levels than allergic patients in the proximal esophagus (1.4 ± 1.1 vs 68.9 ± 20.5 eosinophils/HPF; $P = .004$). Esophageal eosinophil levels in allergic patients did not significantly change with treatment (Figure 5). While there was only a trend toward improved overall responsiveness to FP treatment between nonallergic and allergic patients (70% vs 30%; $P = .18$), nonallergic patients had a significantly better response in the proximal esophagus compared with allergic patients (88% vs 33%; $P = .05$). All nonallergic patients had at least a partial response in the proximal esophagus, compared with 40% of allergic patients who had at least a partial response. When aeroallergen allergic patients were examined, the data were similar. Of patients with aeroallergen allergies treated with FP, only 1 of 6 (17%) responded to therapy, compared with 9 of 14 individuals (64%) without aeroallergen allergies who responded to FP ($P = .14$).

Responsiveness to FP negatively correlates with patient age, height, and weight.

Several patient characteristics were examined to determine their effect on response to FP (Figure 6). FP responders were significantly younger than nonresponders (5.9 ± 0.7 vs 11.3 ± 1.0 years; $P = .0003$). FP responders were also significantly shorter (118.3 ± 3.7 vs 146.2 ± 7.0 cm; $P = .002$) and lighter in weight (23.9 ± 2.3 vs 44.6 ± 7.6 kg; $P = .02$) than nonresponders. There was a trend toward lower body mass index in responders compared with nonresponders (16.8 ± 0.9 vs 19.6 ± 1.6 kg/m²; $P = .15$). Female patients trended toward an improved response rate to FP compared with male patients (75% vs 44%; $P = .58$). There were no differences in duration of symptoms before diagnosis or duration of EE before study enrollment in FP responders compared with nonresponders. There were also no differences in rates of asthma, use of proton pump inhibitors or any acid suppression therapy, or use of leukotriene inhibitors between responders and nonresponders.

Immunopathologic Features

FP lowers esophageal CD8⁺ T-cell levels. Previous reports have shown that there are increased numbers of lymphocytes in the esophageal mucosa of patients with EE relative to normal controls,²⁶ including CD8⁺ lymphocytes.²² Indeed, there was a significant decrease in CD8⁺ T cells with FP treatment (30.2 ± 5.0 pretreatment vs 17.7 ± 5.5 posttreatment CD8⁺ T cells/HPF in the proximal esophagus [$P = .01$] and 36.2 ± 5.7 pretreatment vs 22.8 ± 6.6 posttreatment CD8⁺ T cells/HPF in the distal esophagus [$P = .008$]) (Figure 7). Treatment with placebo had no effect. The reduction in CD8⁺ T cells with FP treatment was limited to FP responders; FP responders had a significantly lower level of CD8⁺ T cells than FP nonresponders after treatment with FP (4.6 ± 0.9 vs 32.2 ± 9.7 CD8⁺ T cells/HPF in the proximal esophagus [$P = .008$] and 7.0 ± 2.0 vs 40.6 ± 10.9 CD8⁺ T cells/HPF in the distal esophagus [$P = .006$]).

FP reduces levels of esophageal mastocytosis.

Recently, esophageal mast cells have been shown to be increased in patients with EE relative to normal controls.^{14,26} In the FP group as a whole, mast cell counts were significantly decreased by treatment (17.1 ± 3.5 pretreatment vs 7.3 ± 2.2 posttreatment mast cells/HPF in the proximal esophagus [$P = .04$] and 17.9 ± 3.1 pretreatment vs 9.8 ± 2.2 posttreatment mast cells/HPF in the distal esophagus [$P = .006$]) and posttreatment mast cell counts were significantly lower in the FP group than in the placebo group (Figure 7). FP responders had significantly lower posttreatment mast cell counts than FP nonresponders (1.8 ± 0.5 vs 13.3 ± 3.6 mast cells/HPF in the proximal esophagus [$P = .004$] and 2.9 ± 1.0 vs 17.5 ± 2.5 mast cells/HPF in the distal esophagus [$P < .0001$]).

Adverse Events

One patient in the FP group developed esophageal candidiasis; this was identified during the follow-up EGD and resolved following 10 days of oral nystatin therapy. There were no other noted side effects or complications attributable to the therapeutic agent.

Discussion

This study showed that swallowed FP induces remission in EE, with histologic resolution noted in approximately

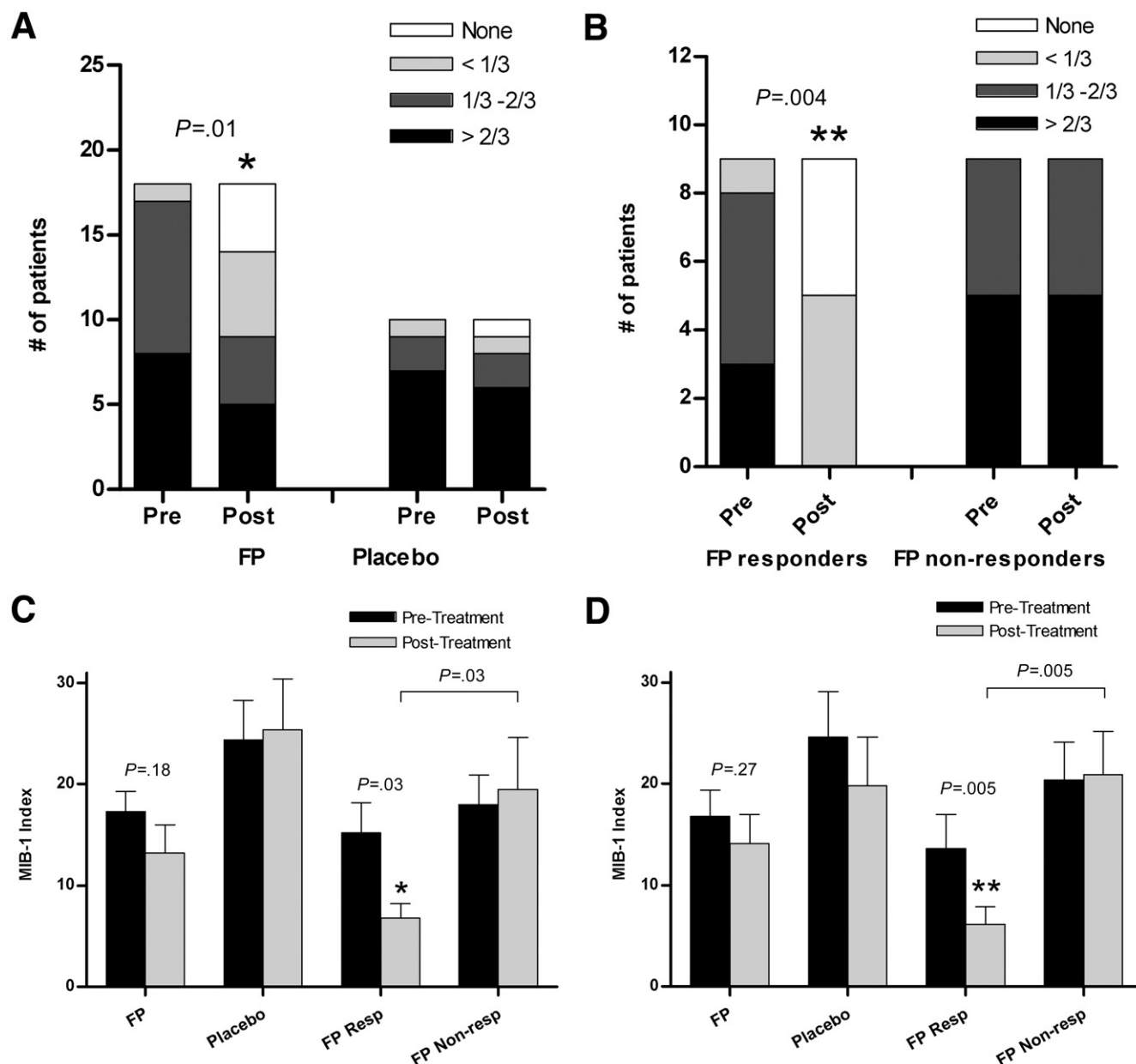


Figure 4. Effect of FP therapy on epithelial hyperplasia in the esophagus. On histologic examination, the thickness of the basal layer in relation to the entire epithelium was estimated in well-oriented sections, and epithelial hyperplasia was graded on a 4-point scale: no hyperplasia, basal layer occupying less than one third of the total epithelial thickness, basal layer occupying one third to two thirds of the total epithelial thickness, and basal layer occupying more than two thirds of the total epithelial thickness. Treatment with FP reduced epithelial hyperplasia in the proximal (not shown) and distal esophagus (A), while placebo had no effect. The reduction in epithelial hyperplasia was limited to FP responders (B). These results were confirmed by immunohistochemical analysis with MIB-1 in the (C) proximal and (D) distal esophagus. * $P < .05$, ** $P < .01$.

50% of patients. FP treatment trended toward greater efficacy in the proximal esophagus, as more patients achieved a complete or partial response in this location than in the distal esophagus. Of note, nearly 50% of patients in the placebo group also had at least a partial response in the proximal esophagus, perhaps indicating that the proximal esophagus is less severely affected by EE or more likely to undergo spontaneous remission than the distal esophagus. More severe pretreatment esophageal eosinophilia (or severity of clinical or endoscopic findings) did not predict poor responsiveness to FP. Improvement in the characteristic endoscopic and histologic features of EE was identified

primarily in those with a concomitant reduction in esophageal eosinophilia; FP nonresponders did not show improvement in these parameters. Treatment with FP significantly reduced layering of eosinophils along the surface of the epithelium. Surface layering of eosinophils correlates with abundant esophageal inflammation and is generally seen only in intensely inflamed biopsy specimens. Resolution of esophageal eosinophilia results in a reduction in surface layering of eosinophils, but this is currently of unclear significance. Vomiting was improved in FP responders. Other clinical symptoms did not change with FP treatment, although this may have been due to the lack of

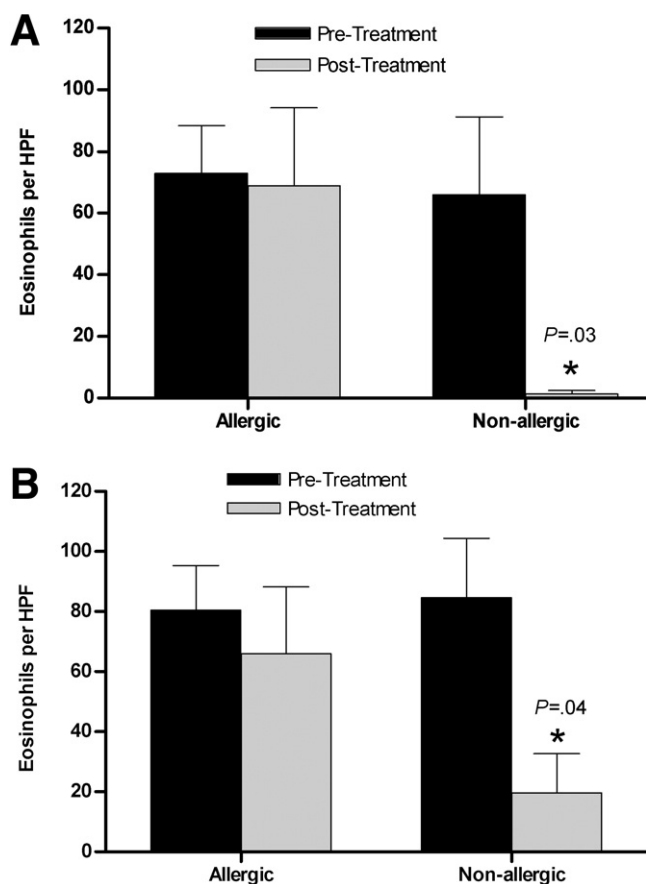


Figure 5. Esophageal eosinophil levels by allergy status in the FP group before and after therapy. *A* and *B* represent the proximal and distal esophagus, respectively. Allergic patients were defined as those with any positive skin prick test result to food and/or environmental allergens. Only nonallergic patients showed a significant decrease in esophageal eosinophil levels with FP treatment. This effect was more pronounced in the proximal esophagus. * $P < .05$.

prospectively collected clinical symptoms for many of the patients. Successful treatment with FP also affected immunopathologic features of EE, reducing both esophageal CD8⁺ T cell and mast cell counts in the esophagus.

The primary end point of the study, histologic remission, was chosen for several reasons. Although there is no universally accepted definition of EE, all current definitions are histologically based, requiring mucosal eosinophilia above a certain threshold, generally a peak eosinophil count greater than 20–24 eosinophils/HPF. Although rare cases of gastroesophageal reflux disease may present with esophageal eosinophilia, it is generally much lower in magnitude than the cutoff used in our study; thus, failure of prior acid suppression therapy was not necessary for a diagnosis of EE in this study. Clinical symptoms may be variable or absent in EE, and no validated clinical scores currently exist. While there are characteristic endoscopic findings in EE, up to 32% of patients have a normal endoscopic appearance of their esophagus.¹² By reducing the burden of inflammatory cells in the esophagus, histologic remission may decrease the potential for long-term complications of chronic inflammation, including fibrosis and stricture formation.²⁷ Because neither clinical symptoms nor endoscopic findings corre-

late well with histologic disease activity, histologic remission served as an objective primary end point.

Previous published reports described nearly universal improvement of EE after treatment with FP,^{8,16} with greater reductions in mucosal eosinophilia and clinical symptoms than the current trial. These studies, however, did not apply a formal definition of remission, only reporting the decrease in mean eosinophil counts with treatment. Using a formal definition of histologic remission (defined as a peak count of ≤ 1 eosinophil/HPF), we recently reported a histologic remission rate of 80% with FP.²³ However, this study was uncontrolled and retrospective, which may have accounted for the greater reported efficacy of FP than in our current study. It is also important to note that in our current study, we did not formally monitor for patient compliance with FP administration; as such, the efficacy of FP may have been partially reduced by any noncompliance.

Nonallergic individuals, as defined by negative skin prick test results to a large panel of food and aeroallergens, had a better response to FP than did allergic individuals, confirming our previous findings.²³ Because allergic patients had already failed or refused an elimination diet, they presumably were still being exposed to either food or aeroallergens to which they were sensitive but which were not identified on skin prick testing. Skin prick testing identifies only immunoglobulin E-mediated reactions, however, and there is evidence that cell-mediated

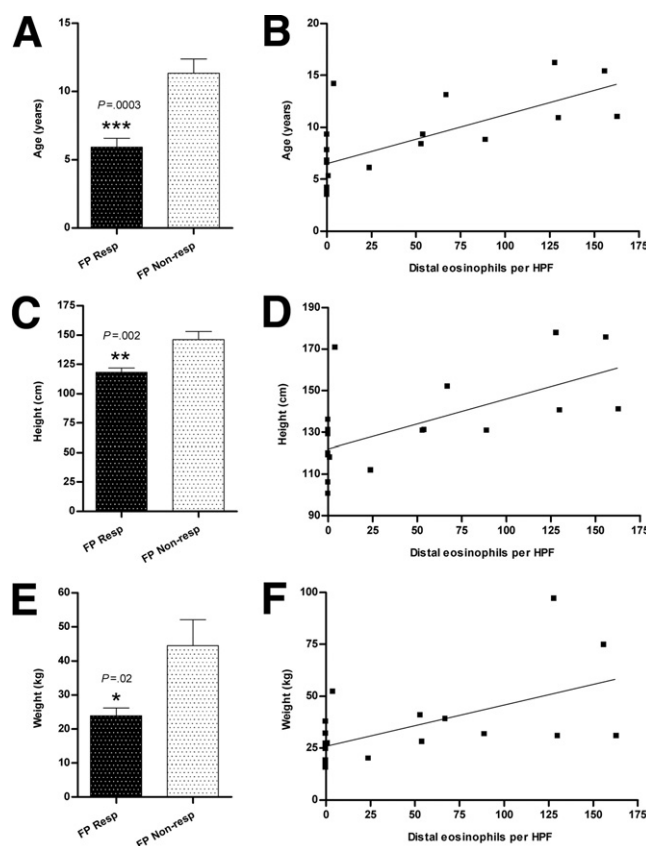


Figure 6. Effect of patient characteristics on response to FP. FP responders were significantly (A) younger, (C) shorter, and (E) lighter in weight than nonresponders. (B) Age, (D) height, and (F) weight significantly correlated with eosinophil levels in the distal esophagus after treatment with FP. * $P < .05$, ** $P < .01$, *** $P < .001$.

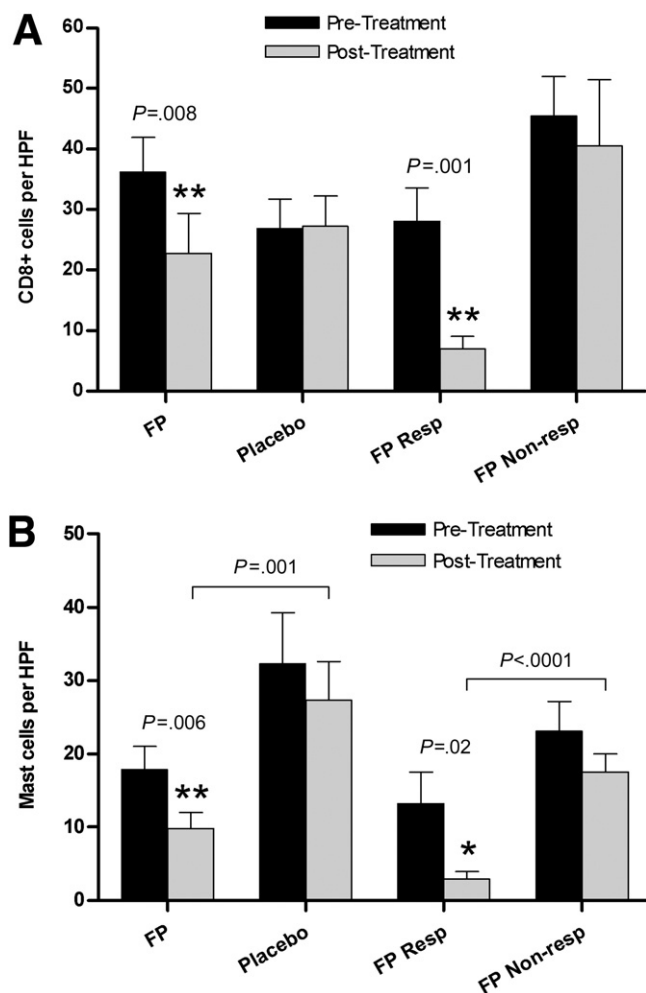


Figure 7. Immunopathologic effects of FP on the esophagus. Treatment with FP decreased esophageal CD8⁺ T cell counts in both the proximal (not shown) and (A) distal esophagus, but this decrease was limited to FP responders. Treatment with FP also decreased esophageal mast cell counts in both the proximal (not shown) and (B) distal esophagus, but this decrease was limited to FP responders. FP responders had lower post-treatment levels of mast cells than FP nonresponders. * $P < .05$, ** $P < .01$.

phenomena may also contribute to the development of EE.²⁸ It is tempting to speculate that aeroallergens may play a more prominent role in EE than previously thought, because 5 of 6 patients (83%) with known aeroallergen sensitivities treated with FP did not respond, similar to the 4 of 6 patients (67%) with food sensitivities who did not respond. These observations are supported by results from murine models of EE, in which esophageal eosinophilia is induced by respiratory antigen challenge.^{15,16} Preliminary studies in humans have supported a role for aeroallergens in EE, with the report of histologically confirmed EE disease exacerbations during pollen seasons in an individual with aeroallergen but not food sensitivities,²⁹ as well as esophageal eosinophil accumulation in patients with allergic rhinitis and known aeroallergen sensitivities.³⁰ Perhaps this ongoing allergen exposure serves as a persistent initiator of inflammation, causing these patients to be refractory to FP therapy. The negative association between responsiveness and allergen sensitization was more pronounced in the proximal

esophagus, as all (10/10) nonallergic individuals had either complete ($n = 8$) or partial ($n = 2$) histologic remission with FP in the proximal esophagus compared with 4 of 10 allergic individuals. This could be due to differential regional esophageal sensitivity to allergens or higher proximal esophageal concentrations of orally ingested allergens. However, it is important to note that the placebo response was higher in nonallergic than in allergic individuals (40% vs 0%). As such, the apparent benefit of FP in nonallergic patients should be interpreted with caution.

To identify factors predicting response to FP, we examined a number of baseline characteristics of FP responders and nonresponders. Responders were significantly younger, shorter, and lighter in weight than nonresponders. While older patients may have had more established disease that was refractory to therapy with FP, there were no differences in disease duration as defined by parental report of symptom onset or endoscopically confirmed EE between FP responders and nonresponders. The mechanism by which swallowed FP acts on esophageal mucosa is believed to be through topical delivery, but this has not been definitively established. Patient height correlates with esophageal length,^{31,32} and because all patients received the same dose of medication, shorter patients would theoretically have received more medication per unit area of esophagus than taller patients. Thus, older or taller patients may require higher doses of FP than younger or shorter patients to induce disease remission. Higher concentrations of FP in the proximal esophagus than in the distal esophagus might also be expected if swallowed FP delivery is primarily topical. There were 3 patients who achieved complete or partial remission in the proximal esophagus without improvement in the distal esophagus, but no patients achieved remission in the distal esophagus without proximal improvement. Other factors may also explain these results, including the lower baseline eosinophil levels in the proximal versus distal esophagus or the lack of universal acid suppression therapy, which could have resulted in residual distal esophageal eosinophilia.

Recent studies have reported increased numbers of mast cells in esophageal biopsy specimens of patients with EE relative to normal controls.^{14,26,33} Esophageal mast cell numbers decreased with FP therapy, primarily in those who achieved histologic remission. Mast cell genes have been shown to be highly induced in EE on microarray analysis, and immunofluorescence microscopy of EE esophageal biopsy specimens suggests involvement of T cell-dependent mucosal mast cells, which may interact with eosinophils and lymphocytes in esophageal mucosa.²⁶ These findings show the importance of delineating the role that mast cells may have in the pathogenesis of EE and further characterizing the potential benefit of anti-mast cell therapy for the treatment of patients with EE.

This study is the first to report a notable placebo effect (9%–18%, depending on the definition of disease remission) that is especially prominent in the proximal esophagus. While the long-term outcome of these patients is not known, this finding has important implications for the natural history of EE, which is only recently beginning to be elucidated.²⁷ Resolution of esophageal eosinophilia in these patients appeared to be spontaneous in as little as 3 months. It will be important to continue to follow these patients, however, to examine the durability of this response. Alternatively, although patients were instructed not to alter their diet or medication regimen during the course

of the study, changes in these parameters may have led to resolution of esophageal eosinophilia. The placebo response in EE deserves further study, because it has bearing on future studies concerning this poorly understood emerging disease.

In conclusion, this study is the first randomized, double-blind, placebo-controlled study concerning therapy for EE. We report that swallowed FP is effective at inducing histologic remission in patients with EE, with a more pronounced effect in nonallergic, younger, shorter, and lighter individuals. In addition, we have shown that FP therapy improves several other aspects of EE, including clinical symptoms, endoscopic appearance, the degree of epithelial hyperplasia, surface layering of eosinophils, and levels of esophageal CD8⁺ T cells and mast cells compared with pretreatment values. With its ease of administration and favorable side effect profile, FP is an attractive therapy for the treatment of patients with EE. It is possible that optimal dosing and improved compliance may increase efficacy, and this and other unresolved issues require additional larger clinical trials to refine the therapeutic role of FP in EE.

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Address requests for reprints to: Marc E. Rothenberg, MD, PhD, Division of Allergy and Immunology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, ML 7028, Cincinnati, Ohio 45229-3039. e-mail: rothenberg@cchmc.org; fax: (513) 636-3310.

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